Assimilation of Glucose Carbon by Various Protein Fractions of Rat Brain and Other Organs

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It has been shown previously that after injection of [U-^{14}C]glucose into intact rat or mouse, the proteins isolated from the whole body, from various organs or from the subcellular components of organ homogenates become rapidly labelled (Vrba, 1962, 1966, 1967; Vrba, Gaitonde & Richter, 1962). The present experiments have shown that a particular group of brain proteins shows a higher rate of incorporation of ^{14}C from [U-^{14}C]glucose than any other group of proteins isolated from brain and liver. Rats were injected with [U-^{14}C]glucose and the content of ^{14}C in proteins of brain, liver, heart and blood was estimated at 30, 60 and 120min. after the injection. The rate of labelling of brain and liver proteins was similar, and was several times higher than the rate of labelling of heart and blood proteins. The proteins of brain and liver were fractionated on the basis of their solubility in water, in sodium deoxycholate and in ammonium sulphate at 72% saturation at pH 7·1. The highest content of ^{14}C was found in brain proteins that were soluble in water and precipitated by 72% saturated ammonium sulphate. These results indicate that virtually all proteins of brain are in a dynamic state of equilibrium, and that the continuous conversion of glucose into protein is an important part of the maintenance of this equilibrium. The metabolic activity of various brain proteins varies significantly; however, no evidence was obtained to indicate the presence of a significant amount of 'metabolically inert protein' in the brain.

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Gel Filtration of [U-^{14}C]Glucose-Labelled High-Speed Supernatants of Rat Brains

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A column of Sephadex G-200 (5cm. wide, 1750ml. total volume) was equilibrated with 0·9% NaCl (flow rate approx. 25ml./hr.) and calibrated with a set of eight non-enzymic protein molecular weight markers (mol.wt. 1450–480000; Mann Research Laboratories Inc., New York, N.Y., U.S.A.). Cube roots of the distribution coefficient were calculated and plotted against the square roots of mol.wt. (Porath, 1963). A satisfactory linear relationship was observed. Six growing rats (60g.) were injected subcutaneously with [U-^{14}C]-glucose (The Radiochemical Centre, Amersham, Bucks.; Batch 141, 2·8mc/m-mole; 56μc in 0·7ml. of 0·9% NaCl/rat). The brains were excised 22hr. later, homogenized in 20ml. of 0·9% NaCl in a Potter–Elvehjem homogenizer for 5min. at 500rev./min. and centrifuged at 38000rev./min. in a Spinco model L, ultracentrifuge with head 40, and the supernatant was subjected to gel filtration on the above column. Blue dextran and D-glucose were added as markers of the void and elution volumes. Then 400 fractions (5ml.) were collected. Protein and ^{14}C were estimated in the eluate as described by Vrba (1967); 99% recovery of ^{14}C was achieved. The temperature during the entire experiment was maintained at 0–2°. The eluate contained a continuous range of proteins with molecular weights ranging from less than 1000 up to more than 800000, and 22hr. after the injection of [U-^{14}C]glucose the labelling of the various proteins separated by gel filtration was nearly uniform.

Similar experiments were performed with intervals of $\frac{1}{4}$, 4, 8, 12 and 92hr. after injection of [^{14}C]glucose, and the specific radioactivities of groups of proteins of various ranges of molecular weights were compared. The proteins eluted from the column were first subjected to a purification procedure for the purpose of determining their specific radioactivities as described by Vrba (1967). Our experiments indicated a metabolic instability of cerebral proteins, and supported similar conclusions by Lajtha & Toth (1966), who used ^{14}C-labelled L-lysine.

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Insulin Secretion and the Intracellular Concentrations of Glucose 6-Phosphate and 6-Phosphogluconate in Isolated Islets of Langerhans

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Evidence has been accumulating that the metabolism of glucose through the pentose phos-